TREATMENT AND DIAGNOSIS OF CANCER

The present application claims the benefit of U.S. Provisional Patent Application 60/022,125, filed July 18, 1996, and is a continuation-in-part of U.S. Patent Application Serial No. 08/838,682, filed April 9, 1997, which claims the benefit of U.S. Provisional Patent Application Serial No. 60/016,976, filed May 6, 1996.

FIELD OF THE INVENTION

The present invention relates to the treatment and diagnosis of cancer with biological agents.

BACKGROUND OF THE INVENTION

In spite of improved treatments for certain forms of cancer, it is still a leading cause of death in the United States. Since the chance for complete remission of cancer is, in most cases, greatly enhanced by early diagnosis, it is very desirable that physicians be able to detect cancers before a substantial tumor develops. However, the development of methods that permit rapid and accurate detection of many forms of cancers continues to challenge the medial community. One such illustrative form of cancer is prostate cancer.

Prostate cancer is the most common cancer in men with an estimated 317,000 cases in 1996 in the United States. It is the second leading cause of death among men who die from neoplasia with an estimated 40,000 deaths per year. Prompt detection and treatment is needed to limit mortality caused by prostate cancer.

Detection of Prostate Cancer

When it metastasizes, prostatic cancer has a distinct predilection for bone and lymph nodes. Saitoh et al., "Metastatic Patterns of Prostatic Cancer: Correlation Between Sites And Number Of Organs Involved," Cancer, 54:3078-3084 (1984). At the time of clinical

diagnosis, as many as 25% of patients have bone metastasis demonstrable by radionuclide scans. Murphy, G.P., et al., "The National Survey Of Prostate Cancer In The United States By The American College Of Surgeons,"

5   J. Urol., 127:928-939 (1982). Accurate clinical evaluation of nodal involvement has proven to be difficult. Imaging techniques such as computed tomography ("CT") or magnetic resonance ("MR") imaging are unable to distinguish metastatic prostate cancer

10 involvement of lymph nodes by criterion other than size (i.e., > 1 cm). Therefore, by definition, these imaging modalities are inherently insensitive in the detection of small volume (< 1 cm) disease as well as non-specific in the detection of larger volume adenopathy. A recent

15 study assessed the accuracy of MR in patients with clinically localized prostate cancer. Rifkin et al., "Comparison Of Magnetic Resonance Imaging And Ultrasonography In Staging Early Prostate Cancer," N. Engel. J. Med., 323:621-626 (1990). In this study, 194

20 patients underwent an MR and 185 of these patients had a lymph node dissection. 23 (13%) patients had pathologically involved lymph nodes. MR was suspicious in only 1 of these 23 cases resulting in a sensitivity of 4%. Similar results have also been noted with CT scans.

25 Gasser et al., "MRI And Ultrasonography In Staging Prostate Cancer," N. Engl. J. Med. (Correspondence), 324(7):49-495 (1991).

The elevation of serum acid phosphatase activity in patients having metastasized prostate

30 carcinoma was first reported by Gutman et al., J. Clin. Invest 17:473 (1938). In cancer of the prostate, prostatic acid phosphatase is released from the cancer tissue into the blood stream with the result that the total serum acid phosphatase level can be greatly

35 increased above normal values. Numerous studies of this enzyme and its relation to prostatic cancer have been made since that time, e.g. Yam, Amer. J. Med. 56:604

(1974). However, the measurement of serum acid phosphatase is elevated in about 65-90 percent of patients having carcinoma of the prostate with bone metastasis; in about 30 percent of patients without

5   roentgenological evidence of bone metastasis; and in about only 5-10 percent of patients lacking clinically demonstrable metastasis.

Prior art attempts to develop a specific test for prostatic acid phosphatase have met with only limited

10   success, because techniques which rely on enzyme activity on a so-called "specific" substrate cannot take into account other biochemical and immunochemical differences among the many acid phosphatases which are unrelated to enzyme activity of prostate origin. In the case of

15   isoenzymes, i.e. genetically defined enzymes having the same characteristic enzyme activity and a similar molecular structure but differing in amino acid sequences and/or content and, therefore, immunochemically distinguishable, it would appear inherently impossible to

20   distinguish different isoenzyme forms merely by the choice of a particular substrate. It is, therefore, not surprising that none of these prior art methods is highly specific for the direct determination of prostatic acid phosphatase activity; e.g. see Cancer 5:236 (1952); J.

25   Lab. Clin. Med. 82:486 (1973); Clin. Chem. Acta. 44:21 (1973); and J. Physiol. Chem. 356:1775 (1975).

In addition to the aforementioned problems of non-specificity which appear to be inherent in many of the prior art reagents employed for the detection of

30   prostate acid phosphatase, there have been reports of elevated serum acid phosphatase associated with other diseases, which further complicates the problem of obtaining an accurate clinical diagnosis of prostatic cancer. For example, Tuchman et al., Am. J. Med. 27:959

35   (1959) noted that serum acid phosphatase levels appear to be elevated in patients with Gaucher's disease.

Due to the inherent difficulties in developing a "specific" substrate for prostate acid phosphatase, several researchers have developed immunochemical methods for the detection of prostate acid phosphatase. However,

5 the previously reported immunochemical methods have drawbacks of their own which have precluded their widespread acceptance. For example, Shulman et al., _Immunology_ 93:474 (1964) described an immuno-diffusion test for the detection of human prostate acid

10 phosphatase. Using antisera prepared from a prostatic fluid antigen obtained by rectal massage from patients with prostatic disease, no cross-reactivity precipitin line was observed in the double diffusion technique against extracts of normal kidney, testicle, liver, and

15 lung. However, this method has the disadvantages of limited sensitivity, even with the large amounts of antigen employed, and of employing antisera which may cross-react with other, antigenically unrelated serum protein components present in prostatic fluid.

20 WO 79/00475 to Chu et. al. describes a method for the detection of prostatic acid phosphatase isoenzyme patterns associated with prostatic cancer which obviates many of the above drawbacks. However, practical problems are posed by the need for a source of cancerous prostate

25 tissue from which the diagnostically relevant prostatic acid phosphatase isoenzyme patterns associated with prostatic cancer are extracted for the preparation of antibodies thereto.

In recent years, considerable effort has been

30 spent to identify enzyme or antigen markers for various types of malignancies with the view towards developing specific diagnostic reagents. The ideal tumor marker would exhibit, among other characteristics, tissue or cell-type specificity. Previous investigators have

35 demonstrated the occurrence of human prostate tissue-specific antigens.

Treatment of Prostate Cancer

As described in W.J. Catalona, "Management of Cancer of the Prostate," New Engl. J. Med.,
5 331(15):996-1004 (1994), the management of prostate cancer can be achieved by watchful waiting, curative treatment, and palliation.

For men with a life expectancy of less than 10 years, watchful waiting is appropriate where low-grade,
10 low-stage prostate cancer is discovered at the time of a partial prostatectomy for benign hyperplasia. Such cancers rarely progress during the first five years after detection. On the other hand, for younger men, curative treatment is often more appropriate.

15 Where prostate cancer is localized and the patient's life expectancy is 10 years or more, radical prostatectomy offers the best chance for eradication of the disease. Historically, the drawback of this procedure is that most cancers had spread beyond the
20 bounds of the operation by the time they were detected. However, the use of prostate-specific antigen testing has permitted early detection of prostate cancer. As a result, surgery is less extensive with fewer complications. Patients with bulky, high-grade tumors
25 are less likely to be successfully treated by radical prostatectomy.

After surgery, if there are detectable serum prostate-specific antigen concentrations, persistent cancer is indicated. In many cases, prostate-specific
30 antigen concentrations can be reduced by radiation treatment. However, this concentration often increases again within two years.

Radiation therapy has also been widely used as an alternative to radical prostatectomy. Patients
35 generally treated by radiation therapy are those who are older and less healthy and those with higher-grade, more clinically advanced tumors. Particularly preferred procedures are external-beam therapy which involves three

dimensional, conformal radiation therapy where the field of radiation is designed to conform to the volume of tissue treated; interstitial-radiation therapy where seeds of radioactive compounds are implanted using

5 ultrasound guidance; and a combination of external-beam therapy and interstitial-radiation therapy.

For treatment of patients with locally advanced disease, hormonal therapy before or following radical prostatectomy or radiation therapy has been utilized.

10 Hormonal therapy is the main form of treating men with disseminated prostate cancer. Orchiectomy reduces serum testosterone concentrations, while estrogen treatment is similarly beneficial. Diethylstilbestrol from estrogen is another useful hormonal therapy which has a

15 disadvantage of causing cardiovascular toxicity. When gonadotropin-releasing hormone agonists are administered testosterone concentrations are ultimately reduced. Flutamide and other nonsteroidal, anti-androgen agents block binding of testosterone to its intracellular

20 receptors. As a result, it blocks the effect of testosterone, increasing serum testosterone concentrations and allows patients to remain potent -- a significant problem after radical prostatectomy and radiation treatments.

25 Cytotoxic chemotherapy is largely ineffective in treating prostate cancer. Its toxicity makes such therapy unsuitable for elderly patients. In addition, prostate cancer is relatively resistant to cytotoxic agents.

30

Use of Monoclonal Antibodies in Prostate Cancer Detection and Treatment

Theoretically, radiolabeled monoclonal

35 antibodies ("mAbs") offer the potential to enhance both the sensitivity and specificity of detecting prostatic cancer within lymph nodes and elsewhere. While many mAbs have previously been prepared against prostate related

antigens, none of these mAbs were specifically generated with an imaging objective in mind. Nevertheless, the clinical need has led to evaluation of some of these mAbs as possible imaging agents. Vihko et al., "Radioimaging of Prostatic Carcinoma With Prostatic Acid Phosphatase - Specific Antibodies," <u>Biotechnology in Diagnostics</u>, 131-134 (1985); Babaian et al., "Radioimmunological Imaging of Metastatic Prostatic Cancer With 111-Indium-Labeled Monoclonal Antibody PAY 276," <u>J. Urol.</u>, 137:439-443 (1987); Leroy et al., "Radioimmunodetection Of Lymph Node Invasion In Prostatic Cancer. The Use Of Iodine 123 (123-I)-Labeled Monoclonal Anti-Prostatic Acid Phosphatase (PAP) 227 A F (ab') 2 Antibody Fragments In Vivo," <u>Cancer</u>, 64:1-5 (1989); Meyers et al., "Development Of Monoclonal Antibody Imaging Of Metastatic Prostatic Carcinoma," <u>The Prostate</u>, 14:209-220 (1989).

In some cases, the monoclonal antibodies developed for detection and/or treatment of prostate cancer recognize antigens specific to malignant prostatic tissues. Such antibodies are thus used to distinguish malignant prostatic tissue (for treatment or detection) from benign prostatic tissue. See U.S. Patent No. 4,970,299 to Bazinet et al. and U.S. Patent No. 4,902,615 to Freeman et al.

Other monoclonal antibodies react with surface antigens on all prostate epithelial cells whether cancerous or benign. See U.S. Patent Nos. 4,446,122 and Re 33,405 to Chu et al., U.S. Patent No. 4,863,851 to McEwan et al., and U.S. Patent No. 5,055,404 to Ueda et al. However, the antigens detected by these monoclonal antibodies are present in the blood and, therefore, compete with antigens at tumor sites for the monoclonal antibodies. This causes background noise which makes the use of such antibodies inadequate for in vivo imaging. In therapy, such antibodies, if bound to a cytotoxic agent, could be harmful to other organs.

Horoszewicz et al., "Monoclonal Antibodies to a New Antigenic Marker in Epithelial Prostatic Cells and Serum of Prostatic Cancer Patients," Anticancer Research, 7:927-936 (1987) ("Horoszewicz") and U.S. Patent No.

5   5,162,504 to Horoszewicz describe an antibody, designated 7E11, which recognizes prostate specific membrane antigen ("PSMA"). Israeli et al., "Molecular Cloning of a Complementary DNA Encoding a Prostate-specific Membrane Antigen," Cancer Research, 53:227-230 (1993) ("Israeli")

10   describes the cloning and sequencing of PSMA and reports that PSMA is prostate-specific and shows increased expression levels in metastatic sites and in hormone-refractory states. Other studies have indicated that PSMA is more strongly expressed in prostate cancer cells

15   relative to cells from the normal prostate or from a prostate with benign hyperplasia. Furthermore, PSMA is not found in serum (Troyer et al., "Detection and Characterization of the Prostate-Specific Membrane Antigen (PSMA) in Tissue Extracts and Body Fluids," Int.

20   J. Cancer, 62:552-558 (1995)).

These characteristics make PSMA an attractive target for antibody mediated targeting for imaging and therapy of prostate cancer. Imaging studies using indium-labeled 7E11 have indicated that the antibody

25   localizes quite well to both the prostate and to sites of metastasis. In addition, 7E11 appears to have clearly improved sensitivity for detecting lesions compared to other currently available imaging techniques, such as CT and MR imaging or bone scan. Bander, "Current Status of

30   Monoclonal Antibodies for Imaging and Therapy of Prostate Cancer," Sem. In Oncology, 21:607-612 (1994).

However, the use of 7E11 and other known antibodies to PSMA to mediate imaging and therapy has several disadvantages. First, PSMA is an integral

35   membrane protein known to have a short intracellular tail and a long extracellular domain. Biochemical characterization and mapping (Troyer et al., "Biochemical

Characterization and Mapping of the 7E11-C5.3 Epitope of
the Prostate-specific Membrane Antigen," <u>Urol. Oncol.</u>,
1:29-37 (1995)) have shown that the epitope or antigenic
site to which the 7E11 antibody binds is present on the
5   intracellular portion of the molecule. Because antibody
molecules do not, under normal circumstances, cross the
cell membrane unless they bind to the extracellular
portion of a molecule and become translocated
intracellularly, the 7E11 antibody does not have access
10   to its antigenic target site in an otherwise healthy,
viable cell.

Consequently, imaging using 7E11 is limited to
the detection of dead cells within tumor deposits.
Additionally, the therapeutic use of the 7E11 antibody is
15   limited, because only cells that are already dead or
tissue containing a large proportion of dead cells can be
effectively targeted.

Although the inadequacies and problems in the
diagnosis and treatment of one particular type of cancer
20   are the focus of the preceding discussion, prostate
cancer is merely a representative model. The diagnosis
and treatment of numerous other cancers have similar
problems.

The present invention is directed to overcoming
25   the deficiencies of prior art antibodies in diagnosing
and treating prostate and other types of cancer.

SUMMARY OF THE INVENTION

30   One aspect of the present invention relates to
a method of ablating or killing cancerous cells. The
process involves providing a biological agent which, when
contacted with an extracellular domain of prostate
specific membrane antigen, recognizes the extracellular
35   domain of prostate specific membrane antigen. These
biological agents are contacted with vascular endothelial
cells proximate to the cancerous cells under conditions

effective to permit both binding of the biological agent to the vascular endothelial cells proximate to the cancerous cells and killing or ablating of the cancerous cells. The biological agent can be used alone or can be

5   bound to a substance effective to kill or ablate the cancerous cells upon binding of the biological agent to vascular endothelial cells that are proximate to the cancerous cells.

In a particularly preferred embodiment of the

10   method of ablating or killing cancerous cells in accordance with the present invention, the biological agent, when contacted with an extracellular domain of prostate specific membrane antigen, binds to and is internalized with the prostate specific membrane antigen

15   of such cells. Preferred biological agents for use in the method of ablating or killing cancerous cells in accordance with the present invention are antibodies or binding portions thereof, probes, or ligands. The methods of the present invention are particularly useful

20   in killing or ablating renal, urothelial, colon, rectal, lung, and breast cancerous cells and cancerous cells of metastatic adenocarcinoma to the liver.

Another aspect of the present invention relates to a method of detecting cancerous tissue in a biological

25   sample. This method involves providing a biological agent which, when contacted with an extracellular domain of prostate specific membrane antigen, binds to the extracellular domain of prostate specific membrane antigen. The biological agent is bound to a label

30   effective to permit detection of vascular endothelial cells proximate to or within the cancerous tissue upon binding of the biological agent to the vascular endothelial cells proximate to or within the cancerous tissue. The biological sample is contacted with the

35   biological agent having a label under conditions effective to permit binding of the biological agent to the vascular endothelial cells proximate to or within the

cancerous tissue in the biological sample. The presence of cancerous tissue in the biological sample is detected by detection of the label.

In a particularly preferred embodiment of the method of detecting cancerous tissue in accordance with the present invention, the biological agent is one that, when contacted with an extracellular domain of prostate specific membrane antigen, binds to and is internalized with the prostate specific membrane antigen. Preferred biological agents for use in the method of detecting cancerous tissue in accordance with the present invention are antibodies or binding portions thereof, probes, or ligands. The method is especially useful in detecting renal, urothelial, colon, rectal, lung, and breast cancerous tissue and cancerous tissue of metastatic adenocarcinoma to the liver.

Still another aspect of the present invention relates to a method of ablating or killing normal, benign hyperplastic, and cancerous prostate epithelial cells. The process involves providing a biological agent which recognizes an extracellular domain of prostate specific membrane antigen. The biological agent can be used alone or can be bound to a substance effective to kill the cells upon binding of the biological agent to the cells. These biological agents are then contacted with the cells under conditions effective to permit both binding of the biological agent to the extracellular domain of the prostate specific membrane antigen and killing or ablating of the cells.

In a particularly preferred embodiment of the method of ablating or killing normal, benign hyperplastic, and cancerous prostate epithelial cells in accordance with the present invention, the biological agent binds to and is internalized with the prostate specific membrane antigen of such cells. Preferred biological agents for use in the method of ablating or killing normal, benign hyperplastic, and cancerous

prostate epithelial cells in accordance with the present invention are antibodies or binding portions thereof, probes, or ligands.

Another aspect of the present invention relates to a method of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells or portions thereof in a biological sample. This method involves providing a biological agent which binds to an extracellular domain of prostate specific membrane antigen. The biological agent is bound to a label effective to permit detection of the cells or portions thereof upon binding of the biological agent to the cells or portions thereof. The biological sample is contacted with the biological agent having a label under conditions effective to permit binding of the biological agent to the extracellular domain of the prostate specific membrane antigen of any of the cells or portions thereof in the biological sample. The presence of any cells or portions thereof in the biological sample is detected by detection of the label.

In a particularly preferred embodiment of the method of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells in accordance with the present invention, the biological agent binds to and is internalized with the prostate specific membrane antigen of such cells. Preferred biological agents for use in the method of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells in accordance with the present invention are antibodies or binding portions thereof, probes, or ligands.

Another aspect of the present invention pertains to a biological agent that recognizes an extracellular domain of prostate specific membrane antigen. In a preferred embodiment, the isolated biological agent binds to and is internalized with the prostate specific membrane antigen. Preferred isolated biological agents which recognize an extracellular domain

of prostate specific membrane antigen in accordance with
the present invention are isolated antibodies or binding
portions thereof, probes, or ligands. Hybridoma cell
lines that produce monoclonal antibodies of these types
5   are also disclosed.

The biological agents of the present invention
recognize the extracellular domain of antigens of normal,
benign hyperplastic, and cancerous prostate epithelial
cells. Unlike the 7E11 antibody, which recognizes an
10 epitope of prostate-associated antigens which are exposed
extracellularly only after cell lysis, the biological
agents of the present invention bind to antigenic
epitopes which are extracellularly exposed in living
prostate cells. Using the biological agents of the
15 present invention, living, unfixed normal, benign
hyperplastic, and cancerous prostate epithelial cells can
be targeted, which makes treatment and diagnosis more
effective. In a preferred embodiment for treating
prostate cancer, the biological agents of the present
20 invention also bind to and are internalized with the
prostate specific membrane antigen, which permits the
therapeutic use of intracellularly acting cytotoxic
agents.

25   **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is an immuno-electron micrograph of
gold-labeled monoclonal antibody J591 on the surface of
LNCaP cells after incubation at 4°C.
30   Figure 2 is an immuno-electron micrograph of
LNCaP cells treated with gold-labeled monoclonal antibody
J591 after 5 minutes incubation at 37°C.
Figure 3 is an immuno-electron micrograph of
LNCaP cells treated with gold-labeled monoclonal antibody
35 J591 after 10 minutes incubation at 37°C.

Figure 4 is an immuno-electron micrograph of LNCaP cells treated with gold-labeled monoclonal antibody J591 after 15 minutes incubation at 37°C.

Figure 5 is an immuno-electron micrograph of LNCaP cells treated with gold-labeled monoclonal antibody J591 after 15 minutes at 37°C showing J591 within endosomes.

Figure 6 summarizes the sequencing strategy of the heavy chain of monoclonal antibody J591.

Figure 7 shows the nucleotide sequence of the heavy chain of monoclonal antibody J591 (designated SEQ.ID. No. 1), the nucleotide sequence of the corresponding reverse, non-coding strand (designated SEQ. ID. No. 2), and the corresponding deduced amino acid sequences (designated SEQ. ID. Nos. 3, 4, and 5).

Figure 8 is a comparison of the heavy chain of monoclonal antibody J591 with the consensus sequence for Mouse Heavy Chains Subgroup IIA.

Figure 9 summarizes the sequencing strategy of the kappa light chain of monoclonal antibody J591.

Figure 10 shows the nucleotide sequences of the kappa light chain of monoclonal antibody J591 (designated SEQ.ID. No. 9), the nucleotide sequence of the corresponding reverse, non-coding strand (designated SEQ. ID. No. 10), and the corresponding deduced amino acid sequence (designated SEQ. ID. Nos. 11, 12, and 13).

Figure 11 is a comparison of the kappa light chain of monoclonal antibody J591 with the consensus sequence for Mouse Kappa Chains Subgroup V.

Figures 12A-12F are micrographs (250 x magnification) showing the immunohistochemical reactivity of mAb J591 to neovasculature of various carcinomas.

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the present invention relates to a method of ablating or killing normal, benign

hyperplastic, and cancerous prostate epithelial cells. The process involves providing a biological agent, such as an antibody or binding portion thereof, probe, or ligand, which binds to an extracellular domain of prostate specific membrane antigen of (i.e., a portion of prostate specific membrane antigen which is external to) such cells. The biological agent can be used alone or can be bound to a substance effective to kill the cells upon binding of the biological agent to the cells. These biological agents are then contacted with the cells under conditions effective to permit both binding of the biological agent to the extracellular domain of the prostate specific membrane antigen and killing or ablating of the cells. In its preferred form, such contacting is carried out in a living mammal by administering the biological agent to the mammal under conditions effective to permit both binding of the biological agent to the extracellular domain of the prostate specific membrane antigen and killing or ablating of the cells. Such administration can be carried out orally or parenterally.

In a particularly preferred embodiment of the method of ablating or killing normal, benign hyperplastic, and cancerous prostate epithelial cells in accordance with the present invention, the biological agent binds to and is internalized with the prostate specific membrane antigen of such cells. Again, the biological agent can be used alone. Alternatively, the biological agent can be bound to a substance effective to kill the cells upon binding of the biological agent to prostate specific membrane antigen and upon internalization of the biological agent with the prostate specific membrane antigen.

The mechanism by which the biological agent is internalized with the prostate specific membrane antigen is not critical to the practice of the present invention. For example, the biological agent can induce

internalization of the prostate specific membrane antigen. Alternatively, internalization of the biological agent can be the result of routine internalization of prostate specific membrane antigen.

5   The above-described biological agents (i.e., biological agents, such as an antibody or binding portion thereof, probe, or ligand which, when contacted with an extracellular domain of prostate specific membrane antigen, recognizes the extracellular domain of prostate

10   specific membrane antigen and, preferably, is internalized therewith) can be used to ablate or kill cancerous cells. In this aspect of the present invention, the biological agent can be used alone or can be bound to a substance effective to kill the cancerous

15   cells upon binding of the biological agent to vascular endothelial cells proximate thereto. These biological agents are contacted with vascular endothelial cells proximate to the cancerous cells. The contacting is carried out under conditions that are effective to permit

20   binding of the biological agent to the vascular endothelial cells proximate to the cancerous cells and, in addition, that are effective to kill or ablate the cancerous cells. The mechanism by which the cancerous cells are killed or ablated is not critical to the

25   practice of the present invention. For example, the cancerous cells can be killed or ablated directly by the biological agent as a consequence of their proximity to the vascular endothelial cells to which the biological agent binds. Alternatively, the biological agent can

30   kill, ablate, or otherwise change the properties of the vascular endothelial cells to which it binds so that blood flow to the cancerous cells proximate thereto is stopped or otherwise reduced, thereby causing the cancerous cells to be killed or ablated. Thus, the

35   method of the present invention is particularly useful for killing or ablating vascular endothelial cells in

cancerous tissue as well as the cancerous cells contained in cancerous tissue.

In a particularly preferred embodiment of the method of ablating or killing cancerous cells in accordance with the present invention, the biological agent employed is one that, when contacted with an extracellular domain of prostate specific membrane antigen, binds to and is internalized with the extracellular domain of prostate specific membrane antigen. The methods of the present invention are particularly useful to kill or ablate cancerous prostate epithelial cells as well as cancerous cells other than cancerous prostate epithelial cells. Examples of cancerous cells which are not cancerous prostate epithelial cells are renal, urothelial, colon, rectal, lung, and breast cancerous cells and cancerous cells of metastatic adenocarcinoma to the liver. Although the method of the present invention can be used to kill or ablate any cell which expresses an extracellular domain of prostate specific membrane antigen or a portion thereof or whose subsistence is dependent upon cells which express an extracellular domain of prostate specific membrane antigen or a portion thereof, the method of the present invention is particularly useful to kill or ablate cancerous cells, because the vascular endothelial cells supplying blood to cancerous tissues (e.g., tumors, collections of cancerous cells, or other cancerous masses) express an extracellular domain of prostate specific membrane antigen, irrespective of the type of cancer involved. In contrast, vascular endothelial cells supplying blood to normal tissues do not express an extracellular domain of prostate specific membrane antigen.

Another aspect of the present invention relates to a method of detecting normal, benign hyperplastic, and cancerous epithelial cells or portions thereof in a biological sample. This method involves providing a

biological agent, such as an antibody or binding portion thereof, probe, or ligand, which binds to an extracellular domain of prostate specific membrane antigen of such cells. The biological agent is bound to a label effective to permit detection of the cells or portions (e.g., prostate specific membrane antigen or fragments thereof liberated from such normal, benign hyperplastic, and cancerous cells) thereof upon binding of the biological agent to the cells or portions thereof. The biological sample is contacted with the biological agent having a label under conditions effective to permit binding of the biological agent to the extracellular domain of the prostate specific membrane antigen of any of the cells or portions thereof in the biological sample. The presence of any cells or portions thereof in the biological sample is detected by detection of the label. In its preferred form, such contacting is carried out in a living mammal and involves administering the biological agent to the mammal under conditions effective to permit binding of the biological agent to the prostate specific membrane antigen of any of the cells or portions thereof in the biological sample. Again, such administration can be carried out orally or parenterally.

The method of the present invention can be used to screen patients for diseases associated with the presence of normal, benign hyperplastic, and cancerous epithelial cells or portions thereof. Alternatively, it can be used to identify the recurrence of such diseases, particularly when the disease is localized in a particular biological material of the patient. For example, recurrence of prostatic disease in the prostatic fossa may be encountered following radical prostatectomy. Using the method of the present invention, this recurrence can be detected by administering a short range radiolabeled antibody to the mammal and then detecting the label rectally, such as with a transrectal detector probe.

Alternatively, the contacting step can be carried out in a sample of serum or urine or other body fluids, such as to detect the presence of PSMA in the body fluid. When the contacting is carried out in a serum or urine sample, it is preferred that the biological agent recognize substantially no antigens circulating in the blood other than PSMA. Since intact prostate cells do not excrete or secrete PSMA into the extracellular environment, detecting PSMA in serum, urine, or other body fluids generally indicates that prostate cells are being lysed. Thus, the biological agents and methods of the present invention can be used to determine the effectiveness of a prostate cancer treatment protocol by monitoring the level of PSMA in serum, urine or other body fluids.

In a particularly preferred embodiment of the method of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells in accordance with the present invention, the biological agent, such as the antibody or binding portion thereof, probe, or ligand, binds to and is internalized with the prostate specific membrane antigen of such cells. Again, the biological agent is bound to a label effective to permit detection of the cells or portions thereof upon binding of the biological agent to and internalization of the biological agent with the prostate specific membrane antigen.

Another aspect of the present invention relates to a method of detecting cancerous tissue in a biological sample. This method involves providing the above-described biological agent (i.e., a biological agent, such as an antibody or binding portion thereof, probe, or ligand which, when contacted with an extracellular domain of prostate specific membrane antigen, recognizes the extracellular domain of prostate specific membrane antigen). The biological agent is bound to a label that is effective to permit detection of vascular endothelial cells proximate to or within the cancerous tissue upon

binding of the biological agent to vascular endothelial cells proximate to or within the cancerous tissue. The biological sample is then contacted with the biological agent having a label. Contacting is carried out under

5 conditions effective to permit binding of the biological agent to the vascular endothelial cells proximate to or within the cancerous tissue in the biological sample. The presence of cancerous cells or portions thereof in the biological sample is detected by detection of the

10 label.

Rather than contacting the entire biological sample with the biological agent, it is contemplated that a portion of the biological sample can be used. For example, a tissue biopsy sample can be contacted with the

15 biological agent to determine the presence of cancerous tissue in the tissue biopsy sample as well as in the larger biological sample from which it is taken. Alternatively, the biological agent can be contacted with a serum or urine sample to acertain whether any vascular

20 endothelial cells expressing an extracellular domain of prostate specific membrane antigen are present therein. Since vascular endothelial cells expressing an extracellular domain of prostate specific membrane antigen are found in the vasculature of cancerous tissues

25 but not in the vasculature of normal tissues, detection of the label in a serum or urine sample indicates the presence of cancerous tissue in the larger biological sample from which it is taken (e.g., a patient).

In a particularly preferred embodiment of the

30 method of detecting cancerous tissues in accordance with the present invention, the biological agent employed is one that, when contacted with an extracellular domain of prostate specific membrane antigen, binds to and is internalized with the prostate specific membrane antigen.

35 The methods of the present invention can be used to detect cancerous prostate epithelial cells as well as cancerous tissues containing cancerous cells other than

cancerous prostate epithelial cells. Examples of cancerous tissues containing cancerous cells other than cancerous prostate epithelial cells which can be detected with the methods of the present invention include renal,
5   urothelial, colon, rectal, lung, and breast cancerous tissue and cancerous tissue of metastatic adenocarcinoma to the liver.

As indicated above, biological agents suitable for either killing, ablating, or detecting cancerous
10   cells and normal, benign hyperplastic, and cancerous prostate epithelial cells include antibodies, such as monoclonal or polyclonal antibodies. In addition, antibody fragments, half-antibodies, hybrid derivatives, probes, and other molecular constructs may be utilized.
15   These biological agents, such as antibodies, binding portions thereof, probes, or ligands, bind to extracellular domains of prostate specific membrane antigens or portions thereof in normal, benign hyperplastic, and cancerous prostate epithelial cells.
20   As a result, when practicing the methods of the present invention to kill, ablate, or detect normal, benign hyperplastic, and cancerous prostate epithelial cells, the biological agents bind to all such cells, not only to cells which are fixed or cells whose intracellular
25   antigenic domains are otherwise exposed to the extracellular environment. Consequently, binding of the biological agents is concentrated in areas where there are prostate epithelial cells, irrespective of whether these cells are fixed or unfixed, viable or necrotic.
30   Additionally or alternatively, these biological agents, such as antibodies, binding portions thereof, probes, or ligands, bind to and are internalized with prostate specific membrane antigens or portions thereof in normal, benign hyperplastic, and cancerous prostate epithelial
35   cells.

Monoclonal antibody production may be effected by techniques which are well-known in the art.

Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*. The

5   antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are

10   cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and

15   practical methodology of fusing such cells is set forth in Kohler and Milstein, Nature 256:495 (1975), which is hereby incorporated by reference.

Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (e.g., a mouse) with the

20   protein or polypeptide of the present invention. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

25   Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol ("PEG") or other fusing agents (See Milstein and Kohler,

30   Eur. J. Immunol. 6:511 (1976), which is hereby incorporated by reference). This immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, including but not limited to rats and humans, is selected to be deficient

35   in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and to have good fusion capability. Many such cell lines are known

to those skilled in the art, and others are regularly
described.

Procedures for raising polyclonal antibodies
are also well known. Typically, such antibodies can be

5 raised by administering the protein or polypeptide of the
present invention subcutaneously to New Zealand white
rabbits which have first been bled to obtain pre-immune
serum. The antigens can be injected at a total volume of
100 μl per site at six different sites. Each injected

10 material will contain synthetic surfactant adjuvant
pluronic polyols, or pulverized acrylamide gel containing
the protein or polypeptide after SDS-polyacrylamide gel
electrophoresis. The rabbits are then bled two weeks
after the first injection and periodically boosted with

15 the same antigen three times every six weeks. A sample
of serum is then collected 10 days after each boost.
Polyclonal antibodies are then recovered from the serum
by affinity chromatography using the corresponding
antigen to capture the antibody. Ultimately, the rabbits

20 are euthenized with pentobarbital 150 mg/Kg IV. This and
other procedures for raising polyclonal antibodies are
disclosed in E. Harlow, et. al., editors, <u>Antibodies: A
Laboratory Manual</u> (1988), which is hereby incorporated by
reference.

25   In addition to utilizing whole antibodies, the
processes of the present invention encompass use of
binding portions of such antibodies. Such binding
portions include Fab fragments, F(ab')$_2$ fragments, and Fv
fragments. These antibody fragments can be made by

30 conventional procedures, such as proteolytic
fragmentation procedures, as described in J. Goding,
<u>Monoclonal Antibodies: Principles and Practice</u>,
pp. 98-118 (N.Y. Academic Press 1983), which is hereby
incorporated by reference.

35   Alternatively, the processes of the present
invention can utilize probes or ligands found either in
nature or prepared synthetically by recombinant DNA

procedures or other biological or molecular procedures.
Suitable probes or ligands are molecules which bind to
the extracellular domains of prostate specific membrane
antigens identified by the monoclonal antibodies of the
5   present invention. Other suitable probes or ligands are
molecules which bind to and are internalized with
prostate specific membrane antigens. Such probes or
ligands can be, for example, proteins, peptides, lectins,
or nucleic acid probes.

10   It is particularly preferred to use the
monoclonal antibodies identified below in Table 1.

TABLE 1

Monoclonal Antibody Name	ATCC Designation for Hybridoma Cell Line
E99	HB-12101
J415	HB-12109
J533	HB-12127
J591	HB-12126

These antibodies can be used alone or as a component in a
mixture with other antibodies or other biological agents
25   to treat cancers or image cancerous tissues (particularly
the vascular endothelial cells therein) or prostate
epithelial cells with varying surface antigen
characteristics.

        Regardless of whether the biological agents are
30   used for treatment or diagnosis, they can be administered
orally, parenterally, subcutaneously, intravenously,
intramuscularly, intraperitoneally, by intranasal
instillation, by intracavitary or intravesical
instillation, intraocularly, intraarterially,
35   intralesionally, or by application to mucous membranes,
such as, that of the nose, throat, and bronchial tubes.
They may be administered alone or with pharmaceutically
or physiologically acceptable carriers, excipients, or
stabilizers, and can be in solid or liquid form such as,

tablets, capsules, powders, solutions, suspensions, or emulsions.

The solid unit dosage forms can be of the conventional type. The solid form can be a capsule, such as an ordinary gelatin type containing the biological agent, such as an antibody or binding portion thereof, of the present invention and a carrier, for example, lubricants and inert fillers such as, lactose, sucrose, or cornstarch. In another embodiment, these compounds are tableted with conventional tablet bases such as lactose, sucrose, or cornstarch in combination with binders like acacia, cornstarch, or gelatin, disintegrating agents such as, cornstarch, potato starch, or alginic acid, and a lubricant like stearic acid or magnesium stearate.

The biological agent of the present invention may also be administered in injectable dosages by solution or suspension of these materials in a physiologically acceptable diluent with a pharmaceutical carrier. Such carriers include sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically and physiologically acceptable carrier, including adjuvants, excipients or stabilizers. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols such as, propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

For use as aerosols, the biological agent of the present invention in solution or suspension may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The materials of the present

invention also may be administered in a non-pressurized form such as in a nebulizer or atomizer.

The biological agents may be utilized to detect cancerous tissues (particularly the vascular endothelial cells therein) and normal, benign hyperplastic, and cancerous prostate epithelial cells *in vivo*. This is achieved by labeling the biological agent, administering the labeled biological agent to a mammal, and then imaging the mammal.

Examples of labels useful for diagnostic imaging in accordance with the present invention are radiolabels such as ^{131}I, ^{111}In, ^{123}I, ^{99}mTc, ^{32}P, ^{125}I, ^{3}H, ^{14}C, and ^{188}Rh, fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as isotopes detectable by short-range detector probes, such as a transrectal probe, can also be employed. These isotopes and transrectal detector probes, when used in combination, are especially useful in detecting prostatic fossa recurrences and pelvic nodal disease. The biological agent can be labeled with such reagents using techniques known in the art. For example, see Wensel and Meares, Radioimmunoimaging and Radioimmunotherapy, Elsevier, New York (1983), which is hereby incorporated by reference, for techniques relating to the radiolabeling of antibodies. See also, D. Colcher et al., "Use of Monoclonal Antibodies as Radiopharmaceuticals for the Localization of Human Carcinoma Xenografts in Athymic Mice", Meth. Enzymol. 121: 802-816 (1986), which is hereby incorporated by reference.

A radiolabeled biological agent of this invention can be used for *in vitro* diagnostic tests. The specific activity of a tagged biological agent, such as a

tagged antibody, binding portion thereof, probe, or
ligand, depends upon the half-life, the isotopic purity
of the radioactive label, and how the label is
incorporated into the biological agent. Table 2 lists

5   several commonly-used isotopes, their specific activities
and half-lives. In immunoassay tests, the higher the
specific activity, in general, the better the
sensitivity.

10   TABLE 2

Isotope	Specific Activity of Pure Isotope (Curies/mole)	Half-Life
^{14}C	6.25×10^1	5720 years
^{3}H	2.01×10^4	12.5 years
^{35}S	1.50×10^6	87 days
^{125}I	2.18×10^6	60 days
^{32}P	3.16×10^6	14.3 days
^{131}I	1.62×10^7	8.1 days

          Procedures for labeling biological agents with
25   the radioactive isotopes listed in Table 2 are generally
known in the art. Tritium labeling procedures are
described in U.S. Patent No. 4,302,438, which is hereby
incorporated by reference. Iodinating, tritium labeling,
and ^{35}S labeling procedures especially adapted for murine
30   monoclonal antibodies are described by Goding, J.W.
(supra, pp 124-126) and the references cited therein,
which are hereby incorporated by reference. Other
procedures for iodinating biological agents, such as
antibodies, binding portions thereof, probes, or ligands,
35   are described by Hunter and Greenwood, Nature 144:945
(1962), David et al., Biochemistry 13:1014-1021 (1974),
and U.S. Patent Nos. 3,867,517 and 4,376,110, which are
hereby incorporated by reference. Radiolabeling elements
which are useful in imaging include ^{123}I, ^{131}I, ^{111}In, and
40   ^{99m}Tc, for example. Procedures for iodinating biological
agents are described by Greenwood, F. et al., Biochem. J.
89:114-123 (1963); Marchalonis, J., Biochem. J.
113:299-305 (1969); and Morrison, M. et al.,

Immunochemistry, 289-297 (1971), which are hereby
incorporated by reference. Procedures for ^{99m}Tc-labeling
are described by Rhodes, B. et al. in Burchiel, S. et al.
(eds.), Tumor Imaging: The Radioimmunochemical Detection

5   of Cancer, New York: Masson 111-123 (1982) and the
references cited therein, which are hereby incorporated
by reference. Procedures suitable for ^{111}In-labeling
biological agents are described by Hnatowich, D.J. et
al., J. Immul. Methods, 65:147-157 (1983), Hnatowich, D.

10 et al., J. Applied Radiation, 35:554-557 (1984), and
Buckley, R. G. et al., F.E.B.S. 166:202-204 (1984), which
are hereby incorporated by reference.

          In the case of a radiolabeled biological agent,
the biological agent is administered to the patient, is

15 localized to the tumor bearing the antigen with which the
biological agent reacts, and is detected or "imaged" in
vivo using known techniques such as radionuclear scanning
using e.g., a gamma camera or emission tomography. See
e.g., A.R. Bradwell et al., "Developments in Antibody

20 Imaging", Monoclonal Antibodies for Cancer Detection and
Therapy, R.W. Baldwin et al., (eds.), pp. 65-85
(Academic Press 1985), which is hereby incorporated by
reference. Alternatively, a positron emission transaxial
tomography scanner, such as designated Pet VI located at

25 Brookhaven National Laboratory, can be used where the
radiolabel emits positrons (e.g., ^{11}C, ^{18}F, ^{15}O, and ^{13}N).

          Fluorophore and chromophore labeled biological
agents can be prepared from standard moieties known in
the art. Since antibodies and other proteins absorb

30 light having wavelengths up to about 310 nm, the
fluorescent moieties should be selected to have
substantial absorption at wavelengths above 310 nm and
preferably above 400 nm. A variety of suitable
fluorescers and chromophores are described by Stryer,

35 Science, 162:526 (1968) and Brand, L. et al., Annual
Review of Biochemistry, 41:843-868 (1972), which are
hereby incorporated by reference. The biological agents

can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Patent Nos. 3,940,475, 4,289,747, and 4,376,110, which are hereby incorporated by reference.

5   One group of fluorescers having a number of the desirable properties described above are the xanthene dyes, which include the fluoresceins derived from 3,6-dihydroxy-9-henylxanthhydrol and resamines and rhodamines derived from 3,6-diamino-9-phenylxanthydrol

10   and lissanime rhodamine B. The rhodamine and fluorescein derivatives of 9-o-carboxyphenylxanthhydrol have a 9-o-carboxyphenyl group. Fluorescein compounds having reactive coupling groups such as amino and isothiocyanate groups such as fluorescein isothiocyanate and

15   fluorescamine are readily available. Another group of fluorescent compounds are the naphthylamines, having an amino group in the α or β position.

Biological agents can be labeled with fluorchromes or chromophores by the procedures described

20   by Goding, J. (supra, pp 208-249). The biological agents can be labeled with an indicating group containing the NMR-active ^{19}F atom, or a plurality of such atoms inasmuch as (i) substantially all of naturally abundant fluorine atoms are the ^{19}F isotope and, thus, substantially all

25   fluorine-containing compounds are NMR-active; (ii) many chemically active polyfluorinated compounds such as trifluoracetic anhydride are commercially available at relatively low cost, and (iii) many fluorinated compounds have been found medically acceptable for use in humans

30   such as the perfluorinated polyethers utilized to carry oxygen as hemoglobin replacements. After permitting such time for incubation, a whole body NMR determination is carried out using an apparatus such as one of those described by Pykett, Scientific American, 246:78-88

35   (1982), which is hereby incorporated by reference, to locate and image cancerous tissues (particularly the

vascular endothelial cells therein) and prostate
epithelial cells.

In cases where it is important to distinguish
between regions containing live and dead prostate
epithelial cells or to distinguish between live and dead
prostate epithelial cells, the antibodies of the present
invention (or other biological agents of the present
invention), labeled as described above, can be
coadministered along with an antibody or other biological
agent which recognizes only living or only dead prostate
epithelial cells labeled with a label which can be
distinguished from the label used to label the subject
antibody. By monitoring the concentration of the two
labels at various locations or times, spatial and
temporal concentration variations of living and dead
normal, benign hyperplastic, and cancerous prostate
epithelial cells can be ascertained. In particular, this
method can be carried out using the labeled antibodies of
the present invention, which recognize both living and
dead epithelial prostate cells, and labeled 7E11
antibodies, which recognize only dead epithelial prostate
cells.

The biological agents can also be utilized to
kill or ablate cancerous cells and normal, benign
hyperplastic, and cancerous prostate epithelial cells *in
vivo*. This involves using the biological agents by
themselves or with a cytotoxic drug to which the
biological agents of the present invention (i.e.,
biological agents recognizing normal, benign
hyperplastic, and cancerous prostate epithelial cells)
are bound. This involves administering the biological
agents bonded to a cytotoxic drug to a mammal requiring
such treatment. In the case of normal, benign
hyperplastic, and cancerous prostate epithelial cells,
since the biological agents recognize prostate epithelial
cells, any such cells to which the biological agents bind
are destroyed. Although such administration may destroy

normal prostate epithelial cells, this is not
problematic, because the prostate is not required for
life or survival. Although the prostate may indirectly
contribute to fertility, this is not likely to be a

5 practical consideration in patients receiving the
treatment of the present invention. In the case of
cancerous tissues, since the biological agents recognize
vascular endothelial cells that are proximate to
cancerous cells, binding of the biological

10 agent/cytotoxic drug complex to these vascular
endothelial cells destroys them, thereby cutting off the
blood flow to the proximate cancerous cells and, thus,
killing or ablating these cancerous cells.
Alternatively, the biological agents, by virtue of their

15 binding to vascular endothelial cells that are proximate
to cancerous cells, are localized proximate to the
cancerous cells. Thus, by use of suitable biological
agents (including those containing substances effective
to kill cells nondiscriminatingly but only over a short

20 range), cells in cancerous tissue (including cancerous
cells) can be selectively killed or ablated.
The biological agents of the present invention
may be used to deliver a variety of cytotoxic drugs
including therapeutic drugs, a compound emitting

25 radiation, molecules of plants, fungal, or bacterial
origin, biological proteins, and mixtures thereof. The
cytotoxic drugs can be intracellularly acting cytotoxic
drugs, such as short-range radiation emitters, including,
for example, short-range, high-energy α-emitters.

30 Enzymatically active toxins and fragments
thereof are exemplified by diphtheria toxin A fragment,
nonbinding active fragments of diphtheria toxin,
exotoxin A (from *Pseudomonas aeruginosa*), ricin A chain,
abrin A chain, modeccin A chain, α-sacrin, certain

35 *Aleurites fordii* proteins, certain Dianthin proteins,
Phytolacca americana proteins (PAP, PAPII and PAP-S),
Morodica charantia inhibitor, curcin, crotin, *Saponaria*

officinalis inhibitor, gelonin, mitogillin, restrictocin, phenomycin, and enomycin, for example. Procedures for preparing enzymatically active polypeptides of the immunotoxins are described in WO84/03508 and WO85/03508,

5 which are hereby incorporated by reference. Certain cytotoxic moieties are derived from adriamycin, chlorambucil, daunomycin, methotrexate, neocarzinostatin, and platinum, for example.

Procedures for conjugating the biological

10 agents with the cytotoxic agents have been previously described. Procedures for conjugating chlorambucil with antibodies are described by Flechner, I,. European Journal of Cancer, 9:741-745 (1973); Ghose, T. et al., British Medical Journal, 3:495-499 (1972); and Szekerke,

15 M., et al., Neoplasma, 19:211-215 (1972), which are hereby incorporated by reference. Procedures for conjugating daunomycin and adriamycin to antibodies are described by Hurwitz, E. et al., Cancer Research, 35:1175-1181 (1975) and Arnon, R. et al. Cancer Surveys,

20 1:429-449 (1982), which are hereby incorporated by reference. Procedures for preparing antibody-ricin conjugates are described in U.S. Patent No. 4,414,148 and by Osawa, T., et al. Cancer Surveys, 1:373-388 (1982) and the references cited therein, which are hereby

25 incorporated by reference. Coupling procedures as also described in EP 86309516.2, which is hereby incorporated by reference.

In a particularly preferred embodiment of the present invention, especially well-suited for killing or

30 ablating normal, benign hyperplastic, and cancerous prostate epithelial cells, a first biological agent is conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug activator is conjugated with a second biological agent

35 according to the present invention, preferably one which binds to a non-competing site on the prostate specific membrane antigen molecule. Whether two biological agents

bind to competing or non-competing binding sites can be
determined by conventional competitive binding assays.
For example, monoclonal antibodies J591, J533, and E99
bind to competing binding sites on the prostate specific

5   membrane antigen molecule. Monoclonal antibody J415, on
the other hand, binds to a binding site which is non-
competing with the site to which J591, J533, and E99
bind. Thus, for example, the first biological agent can
be one of J591, J533, and E99, and the second biological

10 agent can be J415. Alternatively, the first biological
agent can be J415, and the second biological agent can be
one of J591, J533, and E99. Drug-prodrug pairs suitable
for use in the practice of the present invention are
described in Blakely et al., "ZD2767, an Improved System

15 for Antibody-directed Enzyme Prodrug Therapy That Results
in Tumor Regressions in Colorectal Tumor Xenografts,"
Cancer Research, 56:3287-3292 (1996), which is hereby
incorporated by reference.

     Alternatively, the biological agent can be

20 coupled to high energy radiation emitters, for example, a
radioisotope, such as ^{131}I, a γ-emitter, which, when
localized at the tumor site, results in a killing of
several cell diameters. See, e.g., S.E. Order,
"Analysis, Results, and Future Prospective of the

25 Therapeutic Use of Radiolabeled Antibody in Cancer
Therapy", Monoclonal Antibodies for Cancer Detection and
Therapy, R.W. Baldwin et al. (eds.), pp 303-316 (Academic
Press 1985), which is hereby incorporated by reference.
Other suitable radioisotopes include α-emitters, such as

30 ^{212}Bi, ^{213}Bi, and ^{211}At, and β-emitters, such as ^{186}Re and ^{90}Y.
Radiotherapy is expected to be particularly effective,
because prostate epithelial cells and vascular
endothelial cells within cancers are relatively
radiosensitive.

35   Where the biological agents are used alone to
kill or ablate cancerous cells or prostate epithelial
cells, such killing or ablation can be effected by

initiating endogenous host immune functions, such as complement-mediated or antibody-dependent cellular cytotoxicity.

5   The biological agent of the present invention can be used and sold together with equipment, as a kit, to detect the particular label.

Biological agents of the present invention can be used in conjunction with other therapeutic treatment modalities. Such other treatments include surgery,

10   radiation, cryosurgery, thermotherapy, hormone treatment, chemotherapy, vaccines, and other immunotherapies.

Also encompassed by the present invention is a method of killing or ablating which involves using the biological agents for prophylaxis. For example, these

15   materials can be used to prevent or delay development or progression of prostate or other cancers.

Use of the therapeutic methods of the present invention to treat prostate and other cancers has a number of benefits. Since the biological agents

20   according to the present invention only target cancerous cells (such as cells of cancerous tissues containing vascular endothelial cells) and prostate epithelial cells, other tissue is spared. As a result, treatment with such biological agents is safer, particularly for

25   elderly patients. Treatment according to the present invention is expected to be particularly effective, because it directs high levels of biological agents, such as antibodies or binding portions thereof, probes, or ligands, to the bone marrow and lymph nodes where

30   prostate cancer metastases and metastases of many other cancers predominate. Moreover, the methods of the present invention are particularly well-suited for treating prostate cancer, because tumor sites for prostate cancer tend to be small in size and, therefore,

35   easily destroyed by cytotoxic agents. Treatment in accordance with the present invention can be effectively monitored with clinical parameters, such as, in the case

of prostate cancer, serum prostate specific antigen and/or pathological features of a patient's cancer, including stage, Gleason score, extracapsular, seminal, vesicle or perineural invasion, positive margins,

5 involved lymph nodes, etc. Alternatively, these parameters can be used to indicate when such treatment should be employed.

Because the biological agents of the present invention bind to living prostate cells, therapeutic

10 methods for treating prostate cancer using these biological agents are much more effective than those which target lysed prostate cells. For the same reasons, diagnostic and imaging methods which determine the location of living normal, benign hyperplastic, or

15 cancerous prostate epithelial cells (as well as vascular endothelial cells within cancers) are much improved by employing the biological agents of the present invention. In addition, the ability to differentiate between living and dead prostate cells can be advantageous, especially

20 to monitor the effectiveness of a particular treatment regimen.

Hybridomas E99, J415, J533, and J591 have been deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International

25 Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the American Type Culture Collection ("A.T.C.C.") at 12301 Parklawn Drive, Rockville, Maryland 20852. Hybridoma E99 was deposited on May 2, 1996, and received A.T.C.C. Designation Number

30 HB-12101. Hybridoma J415 was deposited on May 30, 1996, and received A.T.C.C. Designation Number HB-12109. Hybridomas J533 and J591 were deposited on June 6, 1996, and received A.T.C.C. Designation Numbers HB-12127 and HB-12126, respectively.

35 The present invention is further illustrated by the following examples.

EXAMPLES

Example 1 -- Human Tissues

Fresh specimens of benign and malignant tissues were obtained from the Department of Pathology of New York Hospital Cornell University Medical Center ("NYH-CUMC").

Example 2 -- Tissue Culture

Cultured cell lines of human cancers were obtained from the Laboratory of Urological Oncology of NYH-CUMC. The prostate cancer cell lines PC-3 (Mickey, D.D., et al., "Characterization Of A Human Prostate Adenocarcinoma Cell Line (DU145) As A Monolayer Culture And As A Solid Tumor In Athymic Mice," Prog. Clin. Biol. Res., 37:67-84 (1980), which is hereby incorporated by reference), DU-145 (Mickey, D.D., et al., "Characterization Of A Human Prostate Adenocarcinoma Cell Line (DU145) As A Monolayer Culture And As A Solid Tumor In Athymic Mice," Prog. Clin. Biol. Res., 37:67-84 (1980), which is hereby incorporated by reference), and LNCaP (Horoszewicz, J.S., et al., "LNCaP Model Of Human Prostatic Carcinoma," Cancer Res., 43:1809-1818 (1983), which is hereby incorporated by reference) were obtained from the American Type Culture Collection (Rockville, MD.). Hybridomas were initially cloned in RPMI-1640 medium supplemented with 10% FCS, 0.1 mM nonessential amino acids, 2mM L-glutamine, 100 units/ml of penicillin, 100 ug/ml of streptomycin and HAT medium (GIBCO, Grand Island, NY). Subclones were cultured in the same medium without aminopterin.

Example 3 -- Preparation of Mouse Monoclonal Antibodies

Female BALB/c mice were immunized intraperitoneally with LNCaP (6×10^6 cells) three times at 2 week intervals. A final intraperitoneal booster immunization was administered with fresh prostate

epithelial cells which had been grown *in vitro*. Three days later, spleen cells were fused with SP-2 mouse myeloma cells utilizing standard techniques (Ueda, R., et al., "Cell Surface Antigens Of Human Renal Cancer Defined

5   By Mouse Monoclonal Antibodies: Identification Of Tissue-Specific Kidney Glycoproteins," Proc. Natl. Acad. Sci. USA, 78:5122-5126 (1981), which is hereby incorporated by reference). Supernatants of the resulting clones were screened by rosette and complement

10   cytotoxicity assays against viable LNCaP. Clones which were positive by these assays were screened by immunochemistry vs normal kidney, colon, and prostate. Clones which were LNCap$^+$/NmlKid$^-$/colon$^-$/prostate$^+$ were selected and subcloned 3 times by limiting dilution. The

15   immunoglobulin class of cultured supernatant from each clone was determined by immunodiffusion using specified rabbit antisera (Calbiochem, San Diego, CA). mAbs were purified using the MAPS-II kit (Bio-Rad, Richmond, CA).

20   Example 4 -- Biotinylation of mAbs

Purified mAbs were dialyzed in 0.1 M NaHCO$_3$ for 2 hours. One ml of mAb at 1 mg/ml was mixed with 0.1 ml of biotinamidocaproate N-hydroxysuccinamide ester (Sigma) in dimethylsulfoxide (1 mg/ml) and stirred for 4 hours at

25   room temperature. Unbound biotin was removed by dialysis against phosphate buffered saline ("PBS").

Example 5 -- Immunohistochemical Staining of Prostate Tissues

30   Cryostat sections of prostate tissues were placed inside rings of Falcon 3034 plate covers (Becton-Dickenson, Lincoln Park, NJ) previously coated with 0.45% gelatin solution as described in Marusich, M.F., "A Rapid Method For Processing Very Large Numbers Of Tissue

35   Sections For Immunohistochemical Hybridoma Screening," J. Immunol. Methods, 111:143-145 (1988), which is hereby incorporated by reference. Plates were stored at -80°C.

Cryostat sections were fixed with 2% paraformaldehyde in PBS for 10 min at room temperature, and, after washing with PBS, endogenous peroxidase activity was blocked by treatment with 0.3% hydrogen peroxide in PBS for 10 min

5   at room temperature. After sections were incubated with 2% BSA in PBS for 20 min, mAbs were added for 60 min at room temperature. Slides were extensively washed with PBS and incubated with peroxidase-conjugated rabbit anti-mouse Ig (DAKO Corp., Santa Barbara, CA) diluted 1:100 in

10   10% normal human serum in PBS for 60 min at room temperature. After a diaminobenzidine reaction, sections were counterstained with hematoxylin.

Example 6 -- Serological Analysis

15   The anti-mouse immunoglobulin mixed hemadsorption assay was performed as described in Ueda, R., et al., "Cell Surface Antigens Of Human Renal Cancer Defined By Mouse Monoclonal Antibodies: Identification Of Tissue-Specific Kidney Glycoproteins," Proc. Natl.

20   Acad. Sci. USA, 78:5122-5126 (1981), which is hereby incorporated by reference. To prepare the indicator cells, anti-mouse Ig (DAKO Corp.) was conjugated to type O human RBC using 0.01% chromium chloride. Serological assays were performed on cells previously

25   plated in Terasaki plates (Nunc, Denmark). Antibodies were incubated with target cells at room temperature for 1 hour. Target cells were then washed, and indicator cells added for 1 hour.

30   Example 7 -- Immunoprecipitation
           LNCaP cells (2 x 10⁷) were biotinylated with biotin-NHSS (at final concentration of 5mM) for 30 minutes on ice. After washing, the biotinylated cells were resuspended in 1 ml lysis buffer (20mM Tris/HCl pH

35   8.0, 1mM EDTA, 1mM PMSF, 1% triton X-100) for 30 min on ice. The suspension was centrifuged at 1500g x 100 min at 4°C, and the supernatant was centrifuged at 12,000 rpm

x 15 min at 4°C. The resulting lysate was preabsorbed with rabbit or goat anti-mouse IgG-coated pansorbin for 1 hour at 4°C. The pre-absorbed lysate was incubated with the mAb overnight at 4°C. Rabbit or goat anti-mouse Ig-
5   coated agarose beads were added for 2 hours at 4°C and then washed. The beads were resuspended in Tris-base/NaCl, added to sample buffer with 2-mercaptoethanol, and boiled for 5 min. After centrifuging, the supernatant was run on an SDS-PAGE 12% gel. The gel was
10 transferred to a nitrocellulose membrane which was blocked and stained with straptavidin-peroxidase. The membrane was developed with diaminobenzidine ("DAB").

Sequential immunoprecipitation was similar except that the lysate was initially pre-cleared with one
15 mAb overnight at 4°C. A second mAb was then used to immunoprecipitate the pre-cleared lysate.

Approximately 2000 clones were screened, of which four clones were selected as described in Example 3, above. After subcloning, supernatants from the 4
20 hybridomas, E99, J415, J533, and J591, were assayed by immunofluorescence against viable (i.e. unfixed) LNCaP, immunoprecipitation, and sequential immunoprecipitation to confirm reactivity to PSMA.

The immunofluorescence study using the LNCaP
25 target cell (described originally in Horoszewicz, which is hereby incorporated by reference, to make the 7E11 antibody and the prototype cell line for expression for PSMA) shows that E99 antibody binds to and renders viable LNCaP cells immunofluorescent. This is in contrast to
30 the 7E11 antibody, which, as noted originally in Horoszewicz, which is hereby incorporated by reference, gives only poor or no binding to viable LNCaP cells but exhibits strong binding once the cells are fixed (killed).

35 The reactivities of the four mAbs with normal human tissues were examined immunohistochemically; these results are presented in Table 3.

TABLE 3

Reactivity of mAbs with human normal tissues
by indirect immunoperosidase staining

Tissues	E99 (γ_3)	J415 (γ_1)	J533 (γ_1)	J591 (γ_1)
Prostate*	●	●	●	●
Kidney				
Glomerulus	o	o	o	o
Prox. Tubule	■	■	■	■
Ureter	o	o	o	o
Bladder	o	o	o	o
Testis	o	o	o	o
Uterus	o			
Esophagus	o	o	o	o
Small Intestine	o	o	o	o
Stomach	o	o	o	o
Colon	o	o	o	o
Spleen	o	o	o	o
Thyroid	o	o	o	o
Lung	o	o	o	o
Pancreas	o	o	o	o
Liver	o	o	o	o
* BPH	0-3⁺	0-3⁺	0-4⁺	0-4⁺
* Prostate Cancer	0-3⁺	0-3⁺	0-4⁺	0-4⁺
* LNCaP (scid)	3⁺	3⁺	4⁺	4⁺
* LuCaP (scid)	0-2⁺	0-2⁺	0-3⁺	0-3⁺

● - positive; ■ - weak, heterogeneous; o - negative

The above sequential immunoprecipitaion study showed that
7E11, E99, J415, J533, and J591 bind to the same
molecule, i.e. PSMA.

Example 8 -- Western Blot Analysis

To confirm that antibodies E99, J415, J533, and
J591 precipitate an identical band to the 7E11 antibody
(i.e., PSMA), Western Blot analyses were performed.
Seminal plasma (400 µg/lane) or LNCaP lysate were loaded
into lanes of 12% SDS-PAGE gels. After electrophoresis,
the gels are transferred to nitrocellulose membranes.
The membranes were blocked with 5% dry milk/Tris-buffered
saline-tween 20 ("TBST") for 60 min at room temperature.
After washing, the membranes were incubated with primary
mAb for 60 min at room temperature. After repeat

washing, the membranes were incubated with sheep anti-mouse-Ig-peroxidase 1/5000 in 5% dry milk/TBST for 60 min at room temperature. After repeat washing, the membranes were developed using a chemiluminescent tag designated

5 "ECL" (Amersham Life Sciences, International, Arlington Heights, Illinois) according to the manufacturer's directions. The results of the Western Blot experiment are presented in Table 4.

10

TABLE 4

Western blot data

Sample	7E11	E99	J415	J533	J591
Prostatic (seminal) fluid	100 KD band	100 KD band	100 KD band	100 KD band	100 KD band
LNCaP cell lysate	100 KD & 200 KD bands	100 KD & 200 KD bands	100 KD & 200 KD bands	100 KD & 200 KD bands	100 KD & 200 KD bands

20

Example 9 -- mAb Reactivity to External Domain of PSMA

To confirm cell surface (external) expression of the detected PSMA, fresh, viable LNCaP cells were

25 tested, without fixation, in vitro, by immunofluorescence. LNCaP cells were washed and incubated with mAb for 1 hour at room temperature and then with a rabbit anti-mouse Ig-fluorescein (DAKO Corp., Santa Barbara, CA). Wells were read with a fluorescent

30 microscope. Negative control consisted of an isotype-matched irrelevant mAb, while an anti-class I MHC mAb served as a positive control.

Immunofluorescence and rosette assay results are presented in Table 5.

TABLE 5

Comparison of 7E11 with new mAbs

	7E11	E99	J415	J533	J591
LNCaP viable cells					
Immunofluor-escence	neg	3+	3+	4+	4+
Rosette assay	neg	+	+	+	+
LNCaP-fixed	+++	++++	+++	++	+++

Example 10 -- Competition Studies

A competition study was carried out to determine whether J591, J533, E99, and J415 detected the same or different antigenic sites (epitopes) of the prostate specific membrane antigen molecule using the following procedure.

Plates were coated with LNCaP cell line lysate as a source of prostate specific membrane antigen and washed to remove unbound material. "Cold" (unlabeled) monoclonal antibody was incubated on the plate for 1 hour at room temperature to allow binding to its antigenic site. Subsequently, a second monoclonal antibody, labeled either with biotin or ^{125}I, was added for an additional hour. Plates were washed to remove unbound material. The amount of the second monoclonal antibody bound to the prostate specific membrane antigen-coated plate was determined either by avidin-alkaline phosphatase in an enzyme-linked immunoassay (in the case of biotin-labeled second monoclonal antibody) or by physically counting the well in a gamma counter (in the case of ^{125}I-labeled second monoclonal antibody). Controls consisted of using the same monoclonal antibody both cold and labeled to define "100% competition" or using monoclonal antibody to a totally different molecule (e.g., monoclonal antibody I-56, which detects inhibin, a

prostate related protein different from prostate specific membrane antigen) to define "0% competition".

The results indicated that J591, J533, and E99 each interfere, compete, or block binding of one another but do not block binding of J415 and vice versa. 7E11/CYT356, known to bind PSMA at a different (intracellular) site, did not block any of J591, J533, E99, or J415.

Having pairs of monoclonal antibodies which bind to non-competing sites permits development of antibody sandwich assays for detecting soluble antigens, such as solubilized prostate specific membrane antigen or fragment thereof, in, for example, body fluids. For example, the antigen (e.g., prostate specific membrane antigen or a fragment thereof) could be "captured" from body fluid with J591 and, in another step, detected by labeled J415.

In another setting, e.g. treatment, one could increase antibody binding by using a combination of non-competing monoclonal antibodies. For example, assuming the non-competing sites are each represented once on the prostate specific membrane antigen molecule, adding a combination of J591 plus J415 would bind twice as many monoclonal antibody molecules as either monoclonal antibody alone. Binding two non-competing antigenic binding sites also can result in greater antigen cross-linking and, perhaps, increased internalization. Furthermore, since the two detected sites are physically located on the same prostate specific membrane antigen molecule, the binding of two monoclonal antibody molecules to that single prostate specific membrane antigen molecule puts the two monoclonal antibody molecules in close proximity to each other, a setting which provides optimal drug-prodrug interaction. For example, monoclonal antibody J591 can be conjugated with an inactive pro-drug and J415 can be conjugated with a pro-drug activator. Since prodrug and activator would be

bound in close proximity only at the site of prostate specific membrane antigen-expressing cells (e.g., prostate cancer cells), prodrug activation to the active form would occur only at those sites.

5

Example 11 -- Microscopy

Confocal microscopy and immuno-electron microscopy demonstrated that E99, J591, J533, and J415 are bound to the cell membrane at clathrin-coated pits and then rapidly internalize into endosomes (cytoplasmic vesicles). Figures 1-4 are immuno-electron micrographs which follow the interaction of gold-labeled monoclonal antibody J591 with the cell surface as a function of time. In these figures, the location of the monoclonal antibody is indicated by the black dots.

Viable LNCaP cells were incubated with J591 for one hour at 4°C. The cells were washed and then held at 37°C for 0, 5, 10, or 15 minutes, after which time they were fixed and processed for immuno-electron microscopy. Figure 1 shows the cell prior to 37°C incubation. J591 can be seen bound to the cell along the external aspect of the cell membrane. In this Figure, "M" denotes the cell's mitochondria, and "N" denotes its nucleus. Figure 2 shows the cell after incubation at 37°C for 5 minutes. The arrow indicates formation of a clathrin-coated pit. In Figure 3, which shows the cell after a 10 minute 37°C incubation, pinching off or endocytosis of the clathrin-coated pit can be seen, as indicated by the arrow. Figure 4 shows that, after incubation at 37°C for 15 minutes, monoclonal antibody J591 is contained in endocytic vesicles within the cell, as indicated by the arrows. As can be seen in Figure 5, after incubation at 37°C for 15 minutes, monoclonal antibody J591 is also contained within endosomes, as indicated by the arrows.

Example 12 -- Sequencing of the Variable Region of Monoclonal Antibody J591

Total RNA was prepared from 10^7 murine hybridoma
5 J591 cells. A sample of the conditioned medium from
these cells was tested for binding to the specific
antigen for J591 on prostate cells. The conditioned
medium was positive by both ELISA and Western Blot for
binding to the antigen.

10 VH and VK cDNA were prepared using reverse
transcriptase and mouse κ constant region and mouse IgG
constant region primers. The first strand cDNAs were
amplified by PCR using a variety of mouse signal sequence
primers (6 for VH and 7 for VK). The amplified DNAs were
15 gel-purified and cloned into the vector pT7Blue.

The VH and VK clones obtained were screened for
correct inserts by PCR, and the DNA sequence of selected
clones was determined by the dideoxy chain termination
method.

20 Excluding the primer region (as the sequence of
this depended on the sequence of the primer that was
used), all the VH clones obtained gave identical
sequence. This sequence was obtained from clones
produced with three different 5' primers. One clone had
25 one base pair change within the signal sequence, and one
clone contained an aberrant PCR product. Using the
sequencing strategy shown in Figure 6, the nucleotide
sequence for the heavy chain was obtained. It is
designated SEQ. ID. No. 1 and is presented in Figure 7,
30 along with the nucleotide sequence of the corresponding
reverse, non-coding strand (designated SEQ. ID. No. 2).
These sequences include part of the signal sequence and
part of the constant region of the antibody. The
corresponding deduced amino acid sequences of J591 VH,
35 designated SEQ. ID. No. 3, SEQ. ID. No. 4, and SEQ. ID.
No. 5, are also shown in Figure 7. The coding strand of
the J591 heavy chain's variable region (exclusive of
signal sequence and constant region components) has the

following nucleotide sequence (designated SEQ. ID. No. 6):

GAGGTCCAGCTGCAACAGTCTGGACCTGAACTGGTGAAGCCTGGGACTTCAGTGAGG
5   ATATCCTGCAAGACTTCTGGATACACATTCACTGAATATACCATACACTGGGTGAAG
CAGAGCCATGGAAAGAGCCTTGAGTGGATTGGAAACATCAATCCTAACAATGGTGGT
ACCACCTACAATCAGAAGTTCGAGGACAAGGCCACATTGACTGTAGACAAGTCCTCC
AGTACAGCCTACATGGAGCTCCGCAGCCTAACATCTGAGGATTCTGCAGTCTATTAT
TGTGCAGCTGGTTGGAACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCC
10   TCA

The reverse, non-coding strand of the J591 heavy chain's variable region (exclusive of signal sequence and constant region components) has the following nucleotide
15   sequence (designated SEQ. ID. No. 7):

TGAGGAGACTGTGAGAGTGGTGCCTTGGCCCCAGTAGTCAAAGTTCCAACCAGCTGC
ACAATAATAGACTGCAGAATCCTCAGATGTTAGGCTGCGGAGCTCCATGTAGGCTGT
ACTGGAGGACTTGTCTACAGTCAATGTGGCCTTGTCCTCGAACTTCTGATTGTAGGT
20   GGTACCACCATTGTTAGGATTGATGTTTCCAATCCACTCAAGGCTCTTTCCATGGCT
CTGCTTCACCCAGTGTATGGTATATTCAGTGAATGTGTATCCAGAAGTCTTGCAGGA
TATCCTCACTGAAGTCCCAGGCTTCACCAGTTCAGGTCCAGACTGTTGCAGCTGGAC
CTC

25   The protein sequence corresponding to the J591 heavy chain's variable region (exclusive of signal sequence and constant region components) has the following nucleotide sequence (designated SEQ. ID. No. 8):

30   EVQLQQSGPELVKPGTSVRISCKTSGYTFTEYTIHWVKQSHGKSLEWIGNINPNNGG
TTYNQKFEDKATLTVDKSSSTAYMELRSLTSEDSAVYYCAAGWNFDYWGQGTTLTVS
S

        The J591 VH is in Mouse Heavy Chains Subgroup
35   IIA (Kabat et al., Sequences of Proteins of Immunological
Interest, U.S. Department of Health and Human Services
(1991) ("Kabat"), which is hereby incorporated by

reference).   The sequence of J591 VH is compared to the consensus sequence for this subgroup in **Figure 8.**

In contrast to the VH, more than one VK sequence was obtained.   Out of the 15 VK clones examined, four gave the sequence of an aberrant mouse Igκ from the fusion partner (Carol et al., Molecular Immunology, 25:991-995 (1988), which is hereby incorporated by reference).   These clones originated from two specific 5' primers.   No further work was done with these clones.   Of the remaining clones, ten gave identical nucleotide sequences, and one clone, VK17, gave an alternative VK sequence.   The ten identical clones originated from three 5' primers (different from the two that gave the aberrant sequence), one of which also produced VK17.   The sequencing strategy that was employed is shown in Figure 9.

The nucleic acid sequence of J591 VK corresponding to the ten identical clones (designated SEQ. ID. No. 9) is presented in Figure 10, along with the nucleic acid sequence of the corresponding reverse, non-coding strand (designated SEQ. ID. No. 10) and the deduced amino acid sequences, which are designated SEQ. ID. No. 11, SEQ. ID. No. 12, and SEQ. ID. No. 13.   These sequences include part of the signal sequence and part of the constant region of the antibody.   The coding strand of the J591 light (kappa) chain's variable region (exclusive of signal sequence and constant region components) corresponding to the ten identical clones has the following nucleotide sequence (designated SEQ. ID. No. 14):

AACATTGTAATGACCCAATCTCCCAAATCCATGTCCATGTCAGTAGGAGAGAGGGTC
ACCTTGACCTGCAAGGCCAGTGAGAATGTGGTTACTTATGTTTCCTGGTATCAACAG
AAACCAGAGCAGTCTCCTAAACTGCTGATATACGGGGCATCCAACCGGTACACTGGG
GTCCCCGATCGCTTCACAGGCAGTGGATCTGCAACAGATTTCACTCTGACCATCAGC
AGTGTGCAGGCTGAAGACCTTGCAGATTATCACTGTGGACAGGGTTACAGCTATCCG
TACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA

The reverse, non-coding strand of the J591 light (kappa) chain's variable region (exclusive of signal sequence and constant region components) corresponding to the ten identical clones has the following nucleotide sequence
5   (designated SEQ. ID. No. 15):

TTTTATTTCCAGCTTGGTCCCCCCTCCGAACGTGTACGGATAGCTGTAACCCTGTCC
ACAGTGATAATCTGCAAGGTCTTCAGCCTGCACACTGCTGATGGTCAGAGTGAAATC
TGTTGCAGATCCACTGCCTGTGAAGCGATCGGGGACCCCAGTGTACCGGTTGGATGC
10   CCCGTATATCAGCAGTTTAGGAGACTGCTCTGGTTTCTGTTGATACCAGGAAACATA
AGTAACCACATTCTCACTGGCCTTGCAGGTCAAGGTGACCCTCTCTCCTACTGACAT
GGACATGGATTTGGGAGATTGGGTCATTACAATGTT

The protein sequence corresponding to the J591 light
15   (kappa) chain's variable region (exclusive of signal sequence and constant region components) corresponding to the ten identical clones has the following nucleotide sequence (designated SEQ. ID. No. 16):

20   NIVMTQSPKSMSMSVGERVTLTCKASENVVTYVSWYQQKPEQSPKLLIYGASNRYTG
VPDRFTGSGSATDFTLTISSVQAEDLADYHCGQGYSYPYTFGGGTKLEIK

The coding strand of the J591 light (kappa) chain's variable region (exclusive of signal sequence and
25   constant region components) corresponding to clone VK17 has the following nucleotide sequence (designated SEQ. ID. No. 17):

GACATTGTGATGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGAGACAGGGTC
30   AGCATCATCTGTAAGGCCAGTCAAGATGTGGGTACTGCTGTAGACTGGTATCAACAG
AAACCAGGACAATCTCCTAAACTACTGATTTATTGGGCATCCACTCGGCACACTGGA
GTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGACTTCACTCTCACCATTACT
AATGTTCAGTCTGAAGACTTGGCAGATTATTTCTGTCAGCAATATAACAGCTATCCT
CTCACGTTCGGTGCTGGGACCATGCTGGACCTGAAA

35

The reverse, non-coding strand of the J591 light (kappa) chain's variable region (exclusive of signal sequence and

constant region components) corresponding to clone VK17
has the following nucleotide sequence (designated SEQ.
ID. No. 18):

5   TTTCAGGTCCAGCATGGTCCCAGCACCGAACGTGAGAGGATAGCTGTTATATTGCTG
     ACAGAAATAATCTGCCAAGTCTTCAGACTGAACATTAGTAATGGTGAGAGTGAAGTC
     TGTCCCAGATCCACTGCCTGTGAAGCGATCAGGGACTCCAGTGTGCCGAGTGGATGC
     CCAATAAATCAGTAGTTTAGGAGATTGTCCTGGTTTCTGTTGATACCAGTCTACAGC
     AGTACCCACATCTTGACTGGCCTTACAGATGATGCTGACCCTGTCTCCTACTGATGT
10   GGACATGAATTTGTGAGACTGGGTCATCACAATGTC

The protein sequence corresponding to the J591 light
(kappa) chain's variable region (exclusive of signal
sequence and constant region components) corresponding to
clone VK17 has the following nucleotide sequence
(designated SEQ. ID. No. 19):

DIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDWYQQKPGQSPKLLIYWASTRHTG
VPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQYNSYPLTFGAGTMLDLK

J591 VK is in the Mouse Kappa Chains Subgroup V
(Kabat, which is hereby incorporated by reference). The
sequence of J591 VK corresponding to the ten identical
clones is compared to the consensus sequence for the
subgroup in Figure 11.

Preferred J591's are those having heavy chain
variable region DNA coding strand sequences corresponding
to SEQ. ID. No. 6 and non-coding strand (reverse)
sequences corresponding to SEQ. ID. No. 7. The heavy
chain variable region of J591 preferably has an amino
acid sequence corresponding to SEQ. ID. No. 8. The light
chain variable region of J591 preferably has a DNA coding
strand sequence corresponding to SEQ. ID. No. 17, a DNA
non-coding strand (reverse) sequence corresponding to
SEQ. ID. No. 18, and a amino acid sequence corresponding
to SEQ. ID. No. 19.

Example 13 -- Immunohistochemical Staining of Normal and Cancer Tissues

Cancer tissues from 23 carcinomas were pre-cooled in liquid nitrogen, snap-frozen in OCT compound (Miles, Elkhart, Indiana) on dry ice, and stored at -80°C. Cryostat tissue sections (5μm) were fixed in cold acetone (4°C) for 10 minutes. mAbs (5 μg/ml or hybridoma supernatants) were incubated for 1 hour at room temperature. Antibody binding was detected using rabbit anti-mouse Ig-peroxidase (Dako, Carpinteria, California) as a secondary antibody and DAB (Sigma, St. Louis, Missouri) as chromogen. Isotype-matched irrelevant antibody was used as negative control.

mAbs J591, J533, J415, and E99 reacted strongly with vascular endothelia in all 23 carcinomas studied, including 9/9 renal, 5/5 urothelial, 6/6 colon, 1/1 lung, and 1/1 breast carcinomas, and 1/1 metastatic adenocarcinoma to the liver. Figures 2A-2F, respectively, show the immunohistochemical reactivity of mAb J591 to neovasculature of renal, urothelial, colon, lung, and breast carcinomas, and metastatic adenocarcinoma to the liver.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose and variations can be made by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.